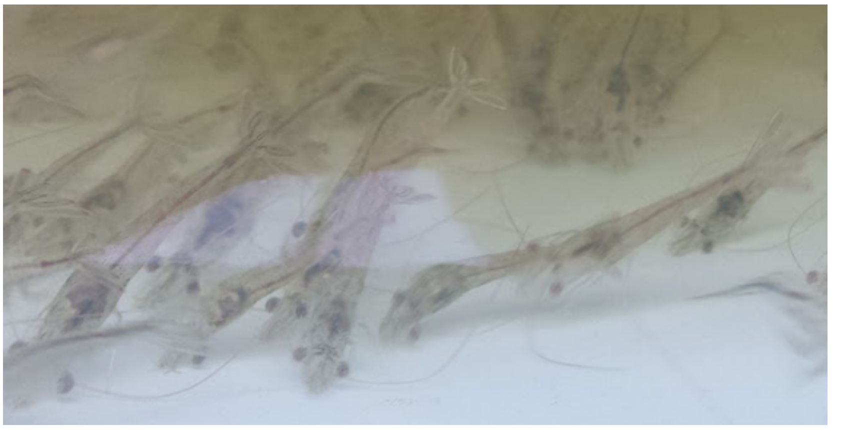

Internship at Auburn University's Fish and Shrimp Nutrition Lab – The Production Cycle of *Litopenaeus vannamei* in Outdoor Ponds and Tank Culture of Trachinotus carolinus

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

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Abstract

The world's population has been on a growing trend for a long time, and it is not expected to slow down in the next decades. It is important that our food production sector can support the nutritional demands current and future expansion will present. FAO data shows a growth in primary productivity over the last decades on both crop and animal production, which has been in response to the increasing food demand.

One of the most import nutrient groups for human consumption is protein, as it plays various and important roles in our physiology and development. Although we can take protein from vegetables, we need a diverse source of protein and other nutrients that are not present in a single food source. This means we will need to continue expanding all aspects of agriculture, which includes fish, which are not only a good source of protein but other valuable nutrients. As more fish stocks have progressively become overfished or fully fished through the years, aquaculture became a focus of great interest. Currently over 60% of the total fish production has its origin in aquaculture.

Regardless of its role in helping fulfill human food requirements, aquaculture is an industry. This means the producers will still look at profit as the final goal. The result of this is a wide range of farmed species allowing for a diverse range of products with differing markets and values. About 62% of the species farmed in aquaculture are fish being most of these freshwater species (FAO, 2016). Crustaceans are economically interesting as they are a relatively large component and have higher final market value. They only account for 8.2% of all live weight traded, but for 21.7% of value traded (FAO, 2016).

With that in mind, this project was designed with the prospect of providing the author the opportunity to participate in the production of marine shrimp using typical production technologies for the Pacific white shrimp (*L. vannamei*) reared under pond production, in typical American conditions. This included, PL accommodation in nursery systems to grow out and harvest in ponds. As part of a nutrition team, this internship also provided the opportunity to experience daily tasks in different nutrition trials in both shrimp (*L. vannamei*) and fish (*T. carolinnus*).

Keywords: Aquaculture, Pond Production, *L. vannamei*, *T. carolinus*, Nutrition, Internship

Resumo

A população mundial tem revelado um crescimento contínuo nas últimas décadas, e não é previsível que esta tendência se inverta num futuro próximo. É por isso importante que o sector primário consiga responder à procura criada pela expansão populacional. Dados da FAO demonstram crescimento da produção primária nas últimas décadas, tanto vegetal como animal, em resposta ao aumento da procura de alimento.

Um dos mais importantes grupos de nutrientes para o Homem são as proteínas, pois desempenham vários papéis em termos fisiológicos e de desenvolvimento. Apesar de ser possível obter proteínas por consumo de vegetais, é vantajoso que todos os nutrientes tenham proveniência de diversas fontes. Significa isto que teremos de continuar a expandir todas as vertentes da agricultura, incluindo a produção de peixe, que não só é uma boa fonte proteica mas de outro nutrientes também. Com o aumento dos stocks em sobrepesca ou completamente explorados, a aquacultura torna-se foco de grande interesse. Atualmente, mais de 60% de todo o pescado produzido é oriundo de aquaculture.

Independentemente do seu papel na satisfação da procura de alimento, a aquacultura é uma indústria. Significa isso que os produtores encaram o lucro como o seu objetivo final. O resultado é uma indústria muito variada capaz de satisfazer vários mercados com diferentes valores. Cerca de 62% das espécies criadas em aquaculture são peixes, a maioria de água doce (FAO, 2016). Os crustáceos são um grupo economicamente interessante pois têm grande valor de mercado. Apesar de só contabilizarem 8,2% de todo o volume de negócio (peso de produção), contabilizam 21,7% de valor transacionado (FAO, 2016)

Este estágio foi, por isso, projetado com vista a proporcionar ao autor a oportunidade de participar na produção de camarão marinho usando tecnologias de produção típicas de camarão branco do Pacífico (*L. vannamei*), produzido em ponds, em condições típicas americanas. Isto inclui desde a aclimatização dos PL em sistemas de berçário à engorda e colheita nos ponds. Como parte de uma equipa de nutrição, este estágio permitiu também conviver com trabalhos diários em ensaios de nutrição tanto em camarão (*L. vannamei*) como em peixe (*T. carolinus*).

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GLOSSARY

CAA – Crystalline Amino Acid

CPC – Corn Protein Concentrate

CPMC – Claude Peteet Mariculture Center

DBY – Dried Bakers Yeast

EAA – Essential Amino Acids

EU – European Union

FAO – Food and Agriculture Organization of United Nations

FCR – Feed Conversion Rate

FM – Fish Meal

Hyp - Hydroxyproline

MBM – Meat Bone Meal

OECD – Organization of Co-operation and Development

PBM – Poultry Byproduct Meal

PL – Post-larval

PUFA – Polyunsaturated Fatty Acids

RAS – Recirculation Aquaculture System

SBM – Soy Bean Meal

SOP – Standard Operating Procedure

UN – United Nations

WSSV – White Spot Syndrome Virus

WWII – World War Two

1. World Population and Protein Requirements

The world population is constantly evolving and changing. Since the Industrial Revolution that started in the late XVIII century, the human population has been on a growing trend. Improved living standards propelled the populations of the countries who benefited from modernization, which in turn then spread to other parts of the world. A gradual change of paradigm contributed to this trend, with science taking the place of religion in the center of western society: Galileo, Newton and Pasteur all lived, worked and published through the Industrial Revolution.

Although the Industrial Revolution represented a period of improvements in public health, it is important to note that sanitation is an old concern of Man. Some civilizations such as the Ancient Greeks and especially the Romans who had already developed some sanitary infrastructures. One example of these structures are roman public baths. However, the Industrial Revolution and its social and scientific repercussions allowed advances in scientific areas such as medicine that would take a key demographic role. Pasteur started a series of discoveries that would be later on referred to as "Golden Age of Microbiology". The discovery of microbes and the advent of vaccines allowed us to gradually understanding disease patterns (to gradually reduce and control disease dissemination) and therefore increase the human lifespan.

Focusing on the past few decades, there is still a growing trend on both world population and average life expectancy. People are expected to continuously grow in numbers and live longer. The UN estimates the growth rate of the human population is about 1.2%. This trend is likely to be maintained as average life expectancy is expected be constantly increasing as investigation in health issues and public health should lead us to a more disease-safe environment. Therefore, the population will gradually increase as a result of higher birth rates, and increased life expectancy. Another important point is the ever-growing development (more or less slow) of basic health in some less developed countries, which may help counter high mortality in the first years of life (Brandt and Gardner, 2000).

This population expansion is a major challenge across many levels of human existence including the basics of food production. How can we produce enough food for everybody? The need to feed the population is not a new problem. Some regions have been suffering from malnutrition for a long time. For millenniums has Mankind been selecting vegetable strains (and animals) that would slowly and gradually answer agricultures needs. As an example, farmers would choose to reproduce cereal plants with more and bigger grains, flowers with more various and uncommon colors, or cows with higher milk production. Nonetheless, further scientific knowledge has fastened this artificial selection process. It helped some regions with production and nutritional problems in various ways, such as creating strains of vegetables that are more resistant to weather, plagues, etc., and with nutrients that normally wouldn't be present in those organisms. The low intake of vitamin A by many Asian populations, for example, has resulted in the development of golden rice, which is enriched with vitamin A (Dawe et al., 2002).

Protein is an important dietary nutrient as it is a primary constituent of animals and it plays a critical role in physiology. Dietary protein serves as a building block for tissues, serves as a source of energy, it is used in the creation of functional chemicals such as enzymes, hormones, antibodies etc. We may ingest proteins from either animal or vegetable sources.

Although we may take proteins from plants, animals are a much denser and more nutritionally complete source of this nutrient. This happens because generally speaking the protein profile of animal muscle is closer to the human necessities. OECD estimates an annual global meat (terrestrial livestock) consumption of 34.1 kg *per capita*, while according to FAO, fish consumption has been on a growth trend and has recently passed the 20 kg per capita mark (FAO, 2016). Although this growth of consumption may have several reasons, it is likely that two of the most important are the growth of producing leading to a descent of prices, as well as an overall consciousness and promotion of the health benefits adjacent to the fish consumption. Fish and fish oil are rich in omega-3 and other PUFA, that play a number of important roles in human health (Sidhu, 2003). Also, ever-growing obesity rates, especially in western countries, make it fairly obvious that we are eating more than ever. Regardless the food source.

Food scarcity is not a new reality to human life. We have lived through a lot of periods of food scarcity and we currently live in a world where the two extremes of feeding are very real: on one side the rich countries face obesity problems, and on the other there are hundreds of people dying every day from malnutrition. But as the world population is expected to continue growing, the problem of feeding everybody will continue to exist until the trend reverses. As the realization of this problem became clearer, we have been trying to find solutions that would allow us to produce even more food.

Meeting our global food demand requires a continuous evolution and growing efficiency of the primary sector. Within the primary sector, the fish production branch has shown great developments in the last decades and is its fastest growing sector

(FAO, 2016). Along with the expansion of production and processing capacity, realizing and promoting of the health benefits of fish consumption is also likely to have played an important role in the increasing of fish consumption. Fish are source of quality protein (good EAA profile) as well as PUFA, vitamins and minerals. Within the fishing sector, one of the industries that has been subject to growing interest is aquaculture.

1.1 Aquaculture Overview

As our animal protein demand increased in the last few decades, one industry that faced major advances was fishing. After WWII, some war technology, such as sonar, started to be put to other uses. The technology once used in naval warfare was then applied to build improved vessels as well as locate and capture big fish schools (Anderson, 1998). This helped expand our fish capture capacity. However, expanding our fishing capacity also led to a growing number of overexploited stocks (FAO, 2016). Ongoing overexploitation eventually leads to the stock collapse and the loss of that resource. Therefore, fisheries are only a renewable resource if we are able to manage it properly. Although the number of overfished stocks has been relatively stable for the last decades (FAO, 2016), the fish capture industry was able to expand its production by turning its attention to underexploited stocks.

With fish captures limited expansion capacity, it has been up to aquaculture to expand global fish production. Aquaculture is the agriculture subsector that sets its focus in the production of aquatic organisms. Although a promising industry, it already plays a determining role in the fish protein production and compliment to wild fisheries. According to FAO, the fish production through captures has been relatively stable for the last 30 years. Hence, aquaculture has expanded to support the continued demand. This has been more pronounced in the same last three decades. Same source states that aquaculture already provides about 60% of the current global fish production (FAO, 2016).

The production of fish has a lot of advantages compared to terrestrial livestock. Aquaculture can produce in three dimensions while cattle are confined to the surface. This allows aquacultures to produce more fish per area unit. Another advantage is that fish are ectothermic which means they barely spend energy maintaining body temperature, so a lot the energy provided in the feed is spent in actual growth. In cattle, an important part of the basal metabolism is spent in keeping the body temperature. Another example of an advantage is the higher protein conversion efficiency, equivalent to that of poultry, and higher than that of swine and cattle.

Along with its many advantages, aquaculture also deals with some typical industrial animal production issues. The two main challenges aquaculture has been facing for the last decades include essentially two categories: **nutrition** and **disease**.

As **nutrition** is concerned, the use of fishmeal in diets is as much as effective as it is expensive, so the industry has been trying to switch fish-based diets for plant-based. The protein and lipid components of fish feeds are gradually switching from fishmeal to plant-based sources. Continuous investigation for the last few decades already allows a complete or partial substitution of protein and lipid components in aquaculture feeds. This substitution is especially important for fish meal and fish oil are an expensive component of feeds, which are the main running cost for farms.

Concerning **disease**, many of the diseases causing major problems in aquaculture are originated by exotic pathogens (Noga, 2010). However, aquaculture stocks fish in higher densities than wild condition, which favors disease outbreak and, consequently, mortality. Disease in high-density farms is usually very hard to control, as parasites, bacteria and virus can spread faster by completing their life cycle in a shorter period of time, especially if they only need one host. This problem is enhanced in case of poor oxygenation as fish or shrimp may be subjected to stressful DO levels, increasing the vulnerability to disease (Noga, 2010). Especially if the species can quickly complete it's life cycle. One of the problems with disease control is the usage of medicine as a prophylactic measure, especially in extensive productions where there is a lack of legislation and control over this activity. This not only favors resistant bacteria to spread but also water pollution downstream the production. Avoiding disease outbreaks, therefore production losses, is also one of the main challenges aquaculture has been facing.

2. Claude Peteet Mariculture Center

The present internship took place at Department of Conservation and Natural Resources, Marine Resources Division, Claude Peteet Mariculture Center in Gulf Shores, Alabama, USA. In addition to a number of Marine Resources Division activities such as marine law enforcement, stock and ecosystem assessment they also maintain the Claude Peteet Mariculture Center (CPMC). This facility serves as the primary business center but also houses aquaculture activities.

Figure 1 – Satellite Image of Claude Peteet Mariculture Center (Source: Google Maps)

For short-term research purposes, Auburn University's fish and shrimp nutrition team makes use of 16 (of the total 35) outside ponds as well as a set of 5 greenhouses. Further description of both ponds and system in greenhouse conditions will be provided further ahead in this report.

3. Learning Objectives and Outcomes

The main objective of this internship is to provide an in-site perspective on standard operating procedures (SOP) for both fish and shrimp culture, as well as how to maintain and deal with real production and research issues. .

By integrating a students field group, it is expected that technical skills are acquired as well as other soft-skills, such as teamwork, team management and, above all, communication skills (in and out of working hours). Communication skills are particularly important when dealing with live animals and complex systems as good communicating is determinant to anticipate or deal with potential problems in production or research systems.

4. *L. vannamei* : Species Background

On this specific project, the main focus is on shrimp production. Namely, Pacific White Shrimp (*Litopenaeus vannamei*), also known as Whiteleg Shrimp. As taxonomy is concerned, it is a decapod (Order: Decapoda), and integrates Dendrobranchiata suborder. It is a warm water marine penaeid shrimp. It is commonly found in the eastern Pacific, from Sonora, Mexico, south to northern Peru (Holthuis, 1980). It is described that larval stages are found in plankton-rich surface waters off-shore, while juvenile prefer brackish waters of estuarine and coastal regions and adults return to higher salinity waters but at higher depth (Lester and Pante, 1992). Therefore, it is, unsurprisingly, capable of withstand a wide range of salinity levels.

Although the first break-through in closing shrimps life cycle occurred on the 1930's (Boyd and Kevin, 2015), and this species is described since at least 1931, the global production only started increasing to considerable numbers in the early 2000's (Bondad-Reantaso, 2012).

Looking from a market perspective, crustaceans have been subject of interest especially on the last decades as it usually has a high market value for both live and frozen sales. Crustacean's high market value is clear if we compare the global production by quantity (live weight) with production by value. Crustaceans account for roughly 8% of global production in terms of quantity but they account for 21.7% in terms of value (FAO, 2016).

Within crustaceans, shrimp are possibly the most important group. In the context of global fish trade, after decades being the most-traded product, shrimp are currently the second, following salmons, trouts and smelts (FAO, 2016). Except for Ecuador, the USA is the main destination for exported shrimp as it not only has been increasing its imports but also declining the country's production (FAO, 2017b).

For its high economical value, wild shrimp resources are highly exploited, particularly in Indian Ocean and Eastern Pacific Ocean, where evidence suggests signs of overexploitation (Bondad-Reantaso, 2012). Shrimp capture by trawl was a very common method of fishing, but the growing awareness of its negative impacts led to a lot of restrictions on its use. This may have also helped boosting shrimp aquaculture.

L. vannamei (Figure 2) has attracted farmers from different regions of the world for it's competitive advantages (comparing to other shrimp species), such as: faster growth rate, safe high stocking density, low salinity tolerance, cold temperature tolerance, less protein requirements, possibility of breeding and domestication, and high disease tolerance (comparing to *P. monodon*) (Bondad-Reantaso et al, 2012; Liu, 2017; Cuzon et al, 2004). In fact, this species has an exceptional capacity to grow in environments with less than ideal conditions (Roy et al., 2010). One example is its capacity to be reared in low salinity ponds. This has been a farming trend in the USA, and *L. vannamei* farming in low salinity will be addressed further ahead (chapter 7).

Figure 2 – Near commercial size *L. vannamei*

These intrinsic farming advantages propelled the species to become the worlds most traded, as shrimp is concerned. In fact, if we look at the provenance of the production in this species there is a clear relationship between the first production growth in the early 1980's and the fact that aquaculture started to be the main product provider to the *L. vannamei* market. Since the year 2000 the wild capture has accounted for less than 1% of the global productivity (FAO, 2017a). Asia is the main producer of *L. vannamei* (over 80% of the global production), followed by America (about 19%) (FAO, 2017a). Although many countries farm this species, most of the biggest productions are in southeast Asia (i.e. China, Thailand, Indonesia, Vietnam) and central America (i.e. Ecuador, Mexico, Brazil, Nicaragua, Honduras) (FAO, 2017c). India is also one of the main global producers with its farming area still expanding (Kumaran et al., 2017).

The fact that shrimp production is a lot of times associated with poor production conditions has led some important buyers to reject shrimp from some countries. One example is the rejection of indian shrimp by the EU Veterenary Authority, and most importantly the American rejection of 133 consignments due to presence of prohibited antibiotics (FAO, 2017a). These prohibitions or tight regulations are likely to push importers to other markets or even, if possible, work around the regulations. Nonetheless, the same source states that the European continent demand for *L. vannamei* has remained low, comparing to other regions and the economic potential of the region.

In a larger timescale, global increase of crustacean production (much related to shrimp aquaculture intensification) and consequent price decrease has allowed an increase of annual per capita availability of crustaceans from 0.4 kg in 1961 to 1.8 kg in 2013 (FAO, 2016). Therefore, it is expected that shrimp aquaculture will continue to increase, following the global food production trends.

5. Shrimp Culture Procedures

Shrimp culture is a complex process that is essentially divided in three stages: hatchery, nursery, and growout. These divisions are related to major changes in the animals conditions. Once the shrimp growing stage is concluded (market size is reached), the ponds or tanks are then harvested and shrimp are ready to be sold or processed before entering the market.

5.1 Hatchery

Following the overall importance of *L. vannamei* for the aquaculture business, and the delicacy of its early stages of production, FAO's Fisheries Department has published a technical paper in 2003 entitled "Health Management and Biosecurity Maintenance in White Shrimp (*Penaeus vannamei*) hatcheries in Latin America". This paper provides technical guidelines for "effective and responsible" operation of shrimp hatcheries. Although it focus on Latin America, most of its guidelines are transversal to all hatcheries, regardless of geographical context.

Hatching is possibly the most delicate part of the production process. This is so in all species but even more in shrimp. This group does not have an acquired immune system (as fish or mammals), therefore broodstock has a very limited capacity of passing immune resistance to descendants. However, domesticated stocks can be bread or genetically improved for trait improvements such as disease resistance, growth and survival. Shrimp hatching consists in three stages: pre-spawning, spawning, and post-spawning. As the quality of larvae will determine the quality of the final product, it is of utmost importance that broodstock, eggs, and hatchlings are under the best water quality and feed conditions. Underestimation of this is likely to result in poor survival and bad final product.

Pre-spawning stage is the selection of individuals in quarantine that both seem to show no sign of disease and/or have been tested for known pathogens. This mitigates as much as possible the possibility of disease introductions into the hatchery system as well as the subsequent spread to the industry. Genetic traits such as poor growth and survival are typical inbreeding issues. This may be solved by cross-breeding stocks from different families or origins, selected from good growth performance ponds or survivors of a disease outbreaks (FAO, 2003). Once screened and acclimated to local conditions they are transferred into the spawning facility.

Spawning stage is characterized by the induction of maturation, spawning and isolation of the eggs. Initially, through maturation period shrimp it is recommended that shrimp may be kept in low densities (6-8 shrimp per m^2), under low-light conditions in controlled photoperiod (10-12 hours dark and 12-14 hours light) and disturbance such as noise should be avoided (FAO, 2003). For water quality is very important through this stage, the same source indicates ideal condition for temperature (28-29 ºC), salinity (30-35 ppt) and pH (8.0-8.2).

High quality feed input and management on broodstock populations is also critical for good maturation, mating, fertility and larvae final quality (Harrison, 1990; Naessens et al., 1997). Although fresh squid, *Artemia*, krill, mussels, clams, etc, are commonly used as broodstock feed (FAO, 2003), polychaetes (mainly *Glycera dibranchiata* and *Americonuphis reseii*) are considered essential for nauplii production (Browdy, 1992), as they are reported to increase reproductive performance (Gomez and Arellano, 1987; Naessens et al., 1997), and it is also reported that PUFA in polychaetes may have a role in promoting maturation (Middleditch et al., 1979; Middleditch et al., 1980; Lytle et al., 1990; Naessens et al., 1997). Due to its high prices, males may be fed cheaper diets (e.g. squid and enriched artificial diets) instead of polychaetes, whenever separated from females (FAO, 2003). The same source states that feeding rates for broodstock should be about 20-30% of biomass (wet weight or frozen).

In domesticated shrimp, the induction of spawning occurs through unilateral ablation of shrimp eyestalk, which is the most common practice for commercial maturation of female penaeid shrimp (Browdy, 1992; Fingerman, 1997; Vaca and Alfaro, 2000). Female eyestalk ablation is a long known and fairly common technique. Male shrimp are capable of mature readily in captivity but unilateral eyestalk ablation increases gonad size and mating frequency (Chamberlain and Lawrence, 1981; Leung-Trujillo and Lawrence, 1985). Although broodstock shrimp may spontaneously mature and spawn in domestic environment, eyestalk ablation helps establishing production cycles through peaks or maturing and spawning (Browdy, 1992; Palacios et al., 1999; Primavera, 1984). The unilateral ablation of eyestalk includes the elimination of the sinus gland, which produces hormones such as gonad- or vitellogenesis-inhibiting hormone (Palacios et al., 1999). This results in lower levels of gonad-inhibiting hormone (GIH), which leads to faster gonad maturation. Unlike the unablated, maturation and spawning peaks are predictable in ablated females (Browdy, 1992; Palacios et al., 1999a; Primavera, 1984). This procedure leads to a faster spawn deterioration (Emmerson, 1980; Primavera, 1984; Tsukimura and Kamemoto, 1991; Vaca and Alfaro, 2000) such as spawning frequency, total nauplii production, and survival to zoea (Palacios et al., 1999b). In result, ablated females should be retired from breeding in period of three months or 15 spawns (FAO, 2003).

Once a female is gravid, it should be transferred to a spawning tank with (one or more) males and as soon as eggs are fertilized they should be kept in suspension using conical bottom tanks and constant aeration to promote hatching (FAO, 2003). Ideal water conditions for optimal hatching are similar to spawning: 29-32 ºC and 32-35 ppt (salinity) (FAO, 2003). The number of eggs spawned per female depends on the shrimp size but for females between 30-45 g it is expected between 100 000 to 200 000 eggs (FAO, 2003). The final fertilization rate should be over 75%, and should it be below 50% then the batch is discarded and investigation should be conducted to assess possible broodstock or system problems (FAO, 2003). Shrimp have, as represented in figure 3, three larval stages (nauplius, zoea, and mysis), followed by one last intermediate stage: post-larvae. Nauplius is the shortest stage (between a day and day and a half) as both zoea and mysis last between 3 and 5 days. The main differences between stages are the growing complexity of organs and increasingly body segmentation. Once the eggs are fertilized, the first larval stage starts its development: nauplius.

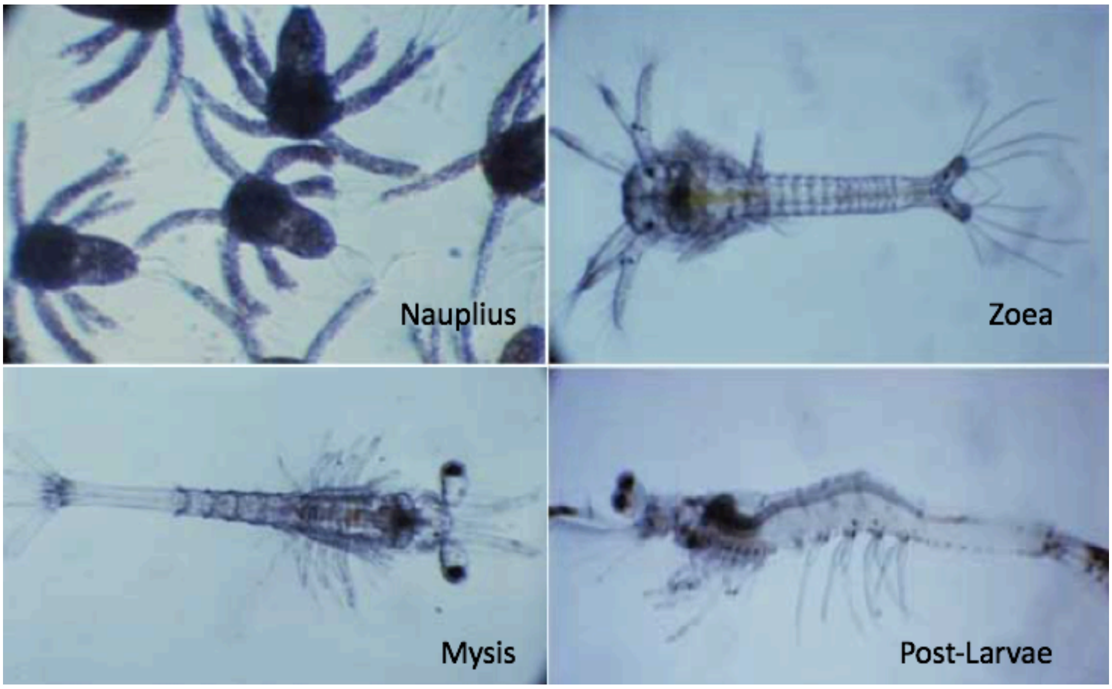

Figure 3 – *L. vannamei* larvae and post-larve stages (Source: Kauai Shrimp)

Post-spawning stage begins when shrimp nauplii are harvested. As with broodstock, poor water quality, feed or health management are very likely to result in poor larvae quality, therefore poor stocks, possibly with higher mortality rates.

Shrimp nauplii harvest is similar to *Artemia* nauplii harvest as in both situations the hatchlings are photosensitive and attracted towards light. Penaeid nauplii should appear in the first 8 hours after egg stocking (FAO, 2003) and rely entirely on yolk for the first 36-48 hours (Quackenbush, 1989). Once nauplii are run out of yolk they are gradually fed from phyto to zooplankton, as they advance in development stages. Both zoea and mysis stages occur through three to five days.

Zoea is the second larval stage and by this point their diet essentially consists in both microalgae and *Artemia* (FAO, 2003). The same source underlines the importance of feeding good quality algae to reduce chances of unsuccessful moult through larval stages as they are particularly stressful due to the ongoing drastic body transformations. By mysis, most structures have already formed, and from post-larvae onwards shrimp gradually evidence fewer differences (except size) between moults.

Through these early stages it is very important to feed them good quality microalgae (e.g. *Chaetocaros*, *Thalassiosira* or *Tetraselmo*) and zooplankton (*Artemia*) for it prevents nutritional related malformation. Through microalgae feeding regime it is particularly important to enhance production biosecurity as *Vibrio spp* will also feed on the same source, therefore may result in disease outbreak (FAO, 2003).

Once the post-larvae development has reached the nursing size it should be stocked in a nursery system. If the PL (post-larvae) are to be sold, they are transported in bags filled with water, oxygen, and *Artemia* as well as activated carbon to reduce ammonia levels and avoid peaks (FAO, 2003). To lower the PL metabolism it is common to reduce the temperature of the transport media.

5.2 Nursery

Shrimp are usually produced under single or multi-phased systems. Single phased systems are typical of extensive and semi-intensive production. In which case the PL are directly introduced in the production ponds. Multi-phased systems acclimate and rear the post-larvae shrimp in nurseries (Figure 4) before the introduction in the production ponds. Such systems are more typical of semi-intensive and intensive production (Samocha and Lawrence, 1992).

For low salinity culture, as in this internship, it is pivotal that shrimp are nursed through decreasing salinity to avoid osmotic shock. For *L. vannamei*, $PL_{<15}$

(post larvae under 15 days old) should not be acclimated to salinity lower than 4 ppt, but $PL_{>15}$ can withstand salinities down to 1 ppt (Davis, 2002). For PL used through this internship the salinity was lowered at an average of 1.64 ppt per day. Although nursery systems may be bypassed in shrimp production, it is suggested that nurseries have a more important and positive role in temperate climates, lower salinity areas and regions with potential disease problems (de Yta et al., 2004). The same author concludes in the same work that nurseries reduce the time shrimp need to be in the growout pond, and that nursery improves not just survival in the ponds, but also size uniformity. The better survival rate may be related to the reduction of time spent in the ponds, as shrimp in nurseries are less vulnerable to disease. Another advantage of nurseries usage is the ease to sample the PL and assess growth, when comparing to directly stocking in the ponds.

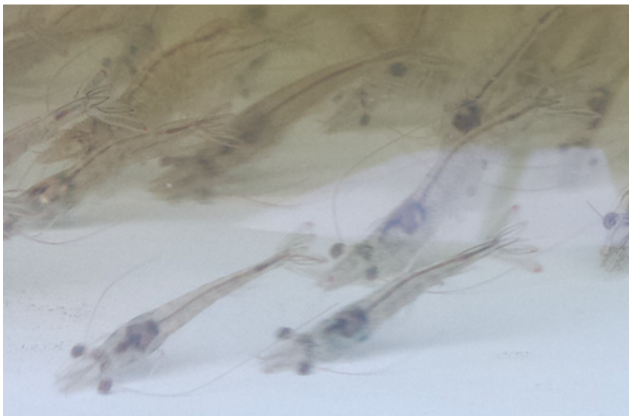

Figure 4 – *L. vannamei* in nursing tank before stocking in experiment system

In conclusion, nurseries advantages are: improved control of counts when stocking growout ponds, better size uniformity at pond harvest, better utilization of farm infrastructures, improved risk management, stronger PL and decreased feed waste (Hirono, 1983; Pretto, 1983; Aquacop, 1985; Fast, 1991; Sturmer et al, 1992; de Yta et al., 2004). The only disadvantage of multi-phased systems is the necessity of building the structure, which is an additional investment for the farmer.

5.3 Growout

The growout stage is the period when shrimp are transferred to outdoor ponds and remain there until harvest. Ponds are very different systems than nurseries. The first major differences are ponds have a natural substrate and a important percentage of dissolved oxygen comes from ponds natural productivity and not mechanical aeration. In the ponds the environment is open so biosecurity is minimal. However, shrimp are already big enough to withstand non-ideal conditions. Especially if they go through a nursing period beforehand and are stocked older and bigger than those that are directly stocked. This lack of biosecurity is compensated by weekly monitoring and health checks.

Through the growout stage, possibly the most important factor to take in consideration is dissolved oxygen (DO). As most farms rear shrimp in intensive and super-intensive densities (above 100 ind/m^2), they tend use mechanical aeration, for natural productivity of the pond cannot provide all the oxygen that these intensive shrimp densities require. As the ponds used in this internship were stocked in semi-intensive densities (38 ind/m^2), we relied more on natural productivity relying on mechanical aeration mostly at night and through algae crashes. Mechanical aeration through the nigh is essential as primary producers synthetize oxygen during the day but, like shrimp, consume oxygen at night. Oxygen availability for shrimp is important as it may be a limitative factor for their growth, and in more extreme situations even survival. In high technology farms (specially ponds under high density rearing) the ponds are usually equipped with real-time DO meters often tied to aeration. These are, constantly immersed in the pond thus allowing for continual oxygen monitoring and more convenient assessment of the necessity of mechanical aeration.

To assure water quality it is essential to understand the oxygen dynamics of the ponds. Particularly, the dynamics related to feeding, as feeding will increase the shrimp activity, therefore oxygen consumption. Weekly monitoring is also important as is allows feed adjustment, which ends up having a positive impact on the farms economy (better resource management) as well as water quality. For this internship a set of rules was established in order to uniform the decision of feeding, or not, a pond. These standard procedures are specified further ahead in this report (sub-chapter 7.1).

5.4 Harvest

Shrimp harvest is the last production stage as it is the process of taking all the shrimp out of the pond for further processing and sale. It is usually scheduled once the shrimp sampling indicates that individuals are within commercial size. In the specific case of *L. vannamei* is it usually between 20 and 25 g (individual weight). Market sized is reached between three and six months depending on culture conditions such as temperature and salinity. However, harvest may be anticipated for reasons such as faster than expected growth or suspect of disease (if shrimp are big enough, to avoid production losses).

The main reasons to harvest earlier are ultimately economical. If growth happens faster than expected, continuing to feed the ponds for an extra period of time may not be economically viable as feed and labour to distribute it is very expensive. Adding to feed cost, shrimp will gradually decrease their growing rate, as carrying capacity of the system is reached and/or they get closer to a much higher size (over 35-40 g). This means the investment in feed will not have a maximum return, as the final price of shrimp doesn't usually compensate the price of feed. Another disadvantage of feeding for an extra period is the oxygen consumption increase in bigger shrimp. This may be a problem in ponds that rely above all in natural productivity, and potentially lead to low oxygen levels that compromise survival. Extensive feeding is also related to water quality degradation related (nitrogen pollution) to feed leaching and waste production. Concerning disease, harvest may be anticipated if one or more ponds are close enough to market size and start showing evidence of disease. In that scenario, to avoid production loss, the affected ponds may be harvested earlier. The process of harvesting a pond starts by draining it. Pond harvest is described ahead in this report on sub-chapter 7.1.

6. Tank Culture of Shrimp

Tank culture typically occurs indoors and allows better biosecurity and control over culture conditions. These systems usually include: production tank, solids removal system, biological filtration, circulation pump(s) for water distribution, aeration system and drainage system. These systems are also called recirculation aquaculture system (RAS). As part of an integrated system, it is pivotal that every component works in tune with the others. Regular maintenance of RAS during nursery and trials and is done by daily syphoning solid waste in the water reservoir and weekly backwashing the systems.

For both nutrition trials as well as nursery tanks recirculation aquaculture systems (RAS) are used. This system design, as the name implies, is a closed system with no water input. The water sanitation is done by a set of two filters: a Bead filter and a biological filter. While the bead filter allows the physical filtration of water, the biological filter metabolizes substances with high toxic-value (ammonia) into other substances through the nitrification process. These substances are nitrite and nitrate and although nitrite is still considerably toxic, it usually does not accumulate in the system as it is promptly converted into nitrate as quickly as it is synthetized (Boyd, 1998). This transformation is possible due to the blooming of nitrifying bacteria, which constitute the biological filter. Regular maintenance of RAS during trials in done by daily syphoning solid waste in the water reservoirs and weekly backwashing the sand filters.

Even with physical and biological treatment, it is important to understand that these systems will also have some natural productivity. This is related to the feed input in the tanks as well as natural light incidence. If the feed input in the tanks is high, then algae and bacteria will be more likely to bloom in the tanks. Algae blooms may also occur if the tanks are exposed to light, especially natural light. This exposure easily (more evident if tanks are not opaque) leads to photosynthetic algae blooms. In outdoor ponds, it is likely that algae blooms occur and they benefit production not only for oxygen production but also because shrimp may eat these algae and or other animals that feed on them. In tank culture, particularly in nutrition requirement trials, it is not desirable to have algae, as the nutritional content cannot be quantified. Within our group we refer to systems as clear water which indicates there is no or very limited levels of natural food sources and green water refer to the presence of algae and natural foods.

For shrimp nutrition trials performed through this internship both systems (clear and green water) were used, in different experiments. These experiments will be addressed further ahead in this report (chapter 10).

6.1 Shrimp Tank Culture Water Quality Management

For water quality management of shrimp tanks (nurseries and experimental systems) the following parameters were controlled: DO, Salinity, pH, Temperature, Ammonia, Nitrite and Nitrate. DO, salinity, pH and temperature were recorded twice a day (0630-0700, 1430-1500) in a YSI multiparametric probe (Yellow Spring Instrument Co., Yellow Spring, OH, USA). Ammonia was registered twice every week, and nitrite and nitrate just once a week. Ammonia was assed by running water samples from every tank through an ISE Multiparameter probe (Thermo Scientific™ Orion™ 4-Star Plus pH/ISE Benchtop Multiparameter Meter) (Figure 5). Nitrite and nitrate were measured through a test kits: Nitrite Nitrogen Tablet Test Kit (LaMotte Company, Chestertown, Maryland, USA) and Nitrate Nitrogen Tablet Test Kit (LaMotte Company, Chestertown, Maryland, USA).

Figure 5 – Water samples and ISE Multiparameter probe

6.2 Shrimp Tank Culture Feed Management

Shrimp feed management can be divided in four different situations: PL nursery, outdoor ponds, nutrition trials and leftover shrimp. Through this internship, two batches of PL were used, in different times. For the first PL were fed both live and inert feed. For the nursing period, PL were initially fed 25% of the estimated biomass but feeding rate was then gradually reduced through this stage. However, for the first six days, this total volume of feed was shared by live (*Artemia*) and inert feed. For the first three days *Artemia* accounted for 50% of total feed and 25% for following three days. Inert feed used in nursery tanks for both batches was Zeigler Raceway Plus (Zeigler Bros Inc., Gardners, PA, USA) with pellet size increasing from #1 to #3 with PL growth. For the second batch, only inert feed was used. Feed amount for PL was determined based on total biomass, which was calculated based on the average weight of 6 groups of 10 PL. Contrary to pond production, for nursery systems no mortality was considered and feed amounts were adjusted based on the groups experience and, if present, the amount of leftover feed in the tanks.

Once stocked, shrimp nutrition trials feeding was performed according to the experiments experimental design. For crystalline amino acid (CAA) trial shrimp daily feed was divided through four meals (0700, 1100, 1500, 1900). For both trials conducted in green water systems, shrimp were fed twice a day (0800, 1600).

7. Shrimp Pond Production

As addressed before, shrimp production is one of the most important sectors of aquaculture's global market as it accounts for over 20% of the annual trade. Although possible to produce indoors, outdoors ponds are the main reservoir for shrimp production. Ponds are a very cheap way of establishing a production site without a big upfront investment. They are possibly the most ancient production systems, but the way farmers approach pond farming has significantly improved. Especially in the last century. These systems are not seen as mere earthen bottom structures dependent on the tides to be filled anymore.

In shrimp pond farming, the main evolution was the knowledge improvement that allowed a production shift in both siting and practices. One good example of early mistakes corrected through time is the typical location of shrimp ponds in mangrove areas and heavy antibiotic dosage as common practice to deal with disease outbreaks (Boyd, 2015). Farming experience through various decades along with multidisciplinary scientific and technological developments enabled farmers to reach its current practices: rear specific-pathogen free animals in high biosecure environment with no water exchange and vegetable-protein feeds, assuring high yields (Boyd, 2015), and located in appropriate sites. With the industrialization of shrimp farming and the increasing of both animal density and feeding rate, ponds are the indicated structure as they allow the blooming of phytoplankton communities. Shrimp will also feed on this natural productivity of the pond.

Although *L. vannamei* has the capacity to grow in less than ideal conditions, most of the industry relies in brackish water for its culture. In fact, most brackish water aquaculture is penaeid shrimp farming (Boyd, 2015). Although assuring higher growth rates (Yan et al, 2007), production in brackish water (15-30 ppt) is much more likely to be struck by disease such as WSSV and several *Vibrio* such as *Vibrio alginolyticus*. High salinity shrimp farming is typical in southeast Asia and production is done under intensive and super intensive densities (100 – 200 shrimp/m^2). Brackish water endemicity, organic pollution and very high animal densities make of these two diseases the most important in *L. vannamei* farming. WSSV and *Vibrio spp* outbreaks usually lead to fast and high mortality, therefore high economical losses.

Contrary to most other areas of the globe such as Southeast Asia and India, it is very common for farms in the south of the USA (Florida, Alabama, Texas, Arizona, etc) to produce shrimp in low salinity (1-15 ppt) (Roy, 2010). Bray et al. (1994) reported no differences in growth rates of shrimp reared in 5 and 15 ppt as well as shrimp reared in 25 and 35 ppt (Ray and Lotz, 2017). In fact, the adaptive capacity of this species is important also from an economical point of view. Firstly because reducing salinity of the environment is an effective treatment against various pathogen agents (Noga, 2010). And secondly, as most brackish water reservoirs located near the coast, it is impractical to pump salt water for long distances. Therefore *L. vannamei*'s capacity to be reared in low salinity allows the farmers to have ponds well away from the sea. This is an advantage for, typically, acreage cost increases with the proximity to the ocean.

7.1 Shrimp Pond Production at CPMC

For shrimp pond production the procedures were based on Van et al. (2017) as the infrastructures and materials used are roughly the same. All 16 ponds used in shrimp production are approximately 0.1 ha in water surface area (46 x 20 x 1 m). Each pond possesses a rectangular catch basin (3.70 x 1.82 x 0.45 m) and a standpipe (20 cm diameter) as well. Pond preparation consisted in the coating of bottom and margins with 1.5 mm thick high polyethylene liner, followed by a bottom coating with ~25 cm of sandy-loam soil. Sediment was tilled prior to filling with brackish water. Pond tilling and liming are common practices between productions cycles to neutralize the acid footprint shrimp production leaves in the ponds bottom. The water inputs in the ponds have a 100% nylon encasement sleeve with a 250 µm mesh to prevent introduction of predatory and larval organisms. Water additions were only done, if necessary, to adjust evaporation losses.

Two weeks before stocking primary productivity in the ponds was promoted by addition of a mixtures of inorganic liquid fertilizers (32-0-0 Ammonium Nitrate and 10-34-0 Ammonium Polyphosphate) at the application rate of 1697 ml and 303 ml, respectively, providing 5.73 kg N and 1.03 kg P_2O_5 per ha. Productivity was regularly assessed by weekly Secchi disk measurements. Through the whole production period oxygen availability was assured by ponds natural productivity and supplemental mechanical aeration (whenever necessary). Each pond was provided a 1-hp aerator (1-hp Aquarian, Air-O-Lator Corporation, Kansas City, MO, USA) to try to keep DO above 3 mg L^{-1} for all ponds, and a second one (1-hp or 2-hp Aire-O2, Aeration Industries International Inc., Minneapolis, MN, USA) whenever necessary.

Previous pond stocking, PL were nursed for a 14 day period. This allowed salinity acclimation to roughly 10 ppt as well as providing other advantages (previously detailed on 5.2 sub-chapter). Pond stocking was performed after assessing weight, and shrimp were transferred from the nurseries to the ponds inside mechanically aerated buckets (Figure 6). To avoid high density and oxygen scarcity stress the ponds transportation was performed twice.

Figure 6 – System used to transfer shrimp from nurseries to ponds

Once the ponds were stocked, the production runs according to a variety of standard procedures, according to the ponds conditions and their dynamics. Daily permanent pond work consisted in measuring water quality parameters (addressed with more detail further ahead in this report) and make decisions on feeding management and artificial aeration. To standardize procedures between students, a set of rules was set. Feeding is skipped if DO measurement indicates a value bellow 2 mg L^{-1}, and mechanical aeration is turned on if bellow 3 mg L^{-1}. Supplemental mechanical aeration (second aerator) is turned on in the pond if DO is lower than 1 mg L^{-1} during the day, or lower than 3 mg L^{-1} at sunset (1930-2000). During the first half of the production cycle night time aeration is run on as needed whereas during the second half mechanical aeration is constant during night period.

As shrimp growth progresses and feeding rates increase, ponds natural productivity also takes advantage of the feed input in ponds and population expansions are more likely. Algae population bloom and collapse may be observed by checking the ponds color. If the water in a pond is brown or foam is present, it is likely that the photosynthetic community is crashing, which may lead to low DO levels. This is important, as pond color is also an indicator of its condition. Feeding increases shrimp metabolism, leading to more oxygen consumption. This information is important to consider when adjusting daily feed inputs and DO levels (i.e. if DO is low and the pond phytoplankton appears to be crashing) it may be best to stop feeding to reduce DO demands. This is to say: although there is a set of standard rules to follow, pond dynamics and condition ultimately determine aeration and feeding decisions. Through the final weeks of the growout stage, high mortality happened in one of the ponds under the higher feeding rate. This was due to various factors but generally happened by initiating automatic feeding in a pond still recovering from algae crash. As the ponds primary productivity couldn't keep up with the high oxygen consumption of big shrimp feeding, DO quickly fell. Poor communication on the feeding situation of the pond delayed an effective response. This resulted in high mortality due to shrimp exposure to very low DO (<1 mg L^{-1}) for a period time of a few hours.

Throw growout, shrimp in the ponds were fed "Semi intensive SI-35%P 7%L 2.4 mm" by Zeigler (Zeigler Bros Inc., Gardners, PA, USA). This is a plant-based diet manufactured with plant protein, grain and grain by-products, yeast and yeast by-products. Due to its high level of plant and plant by-products incorporation it is formulated with low levels of phosphorous to ensure better pond conditions (water quality). Phosphorous is particularly important in shrimp culture for it is associated with eutrophication and *L. vannmaei* has higher requirement (1.33%) for this mineral than most fish (0.3-1.5%) (NRC, 2011; Lall, 2012; Pan et al., 2005). The main difference between diet constitution from nursery to pond culture is a decreasing percentage of protein and fat content, compensated by increased fiber and ash content. Pond feed management was performed according to the experiment presented in subchapter 10.1.1.

Through growout weekly samplings are performed in order to assess shrimp growth. Pond sampling consisted in the capture and weighing of groups of over 60 shrimp. Shrimp capture was performed by cast-net. Individual weight calculated every week served to assess growth, adjust feed and evaluate shrimp general condition. Keeping an updated record of shrimp growth allows better management of production. Due to faster growing rate than expected, shrimp harvest was completed three week earlier. Primary consideration for early harvest were: a) all shrimp would be market size (>20 g), b) all treatments were statistically different, but most importantly c) the high feed inputs of one of the treatments was pushing the aeration capacity of the facility. DO is such a pivotal necessity for shrimp that during the last two weeks of production one last check was performed at midnight (0000) to confirm that mechanical aeration was working properly. In an industrialized farm, ponds are commonly under constant monitoring by underwater probes and any major water quality variation or component malfunction is promptly reported and night working crew will fix the problem.

Once shrimp are ready to harvest it is important to make sure all the logistics are ready for shrimp harvest, euthanize, and conservation until transportation to processing building. Gradual pond draining starts a few days before so that by the eve of harvest all ponds are about 25% of total water volume. The ponds are completely drained individually as they are to be harvested. This remaining water is pumped out of the ponds through a fish pump (Aqualife – Life pump Magic Valley Heli-arc and Mfg, Twin Falls, ID, USA) placed in the catch basin (Figure 7). As water volume is reduced, more shrimp are pumped. Water and shrimp are pumped to a harvester. Remaining shrimp in the pond are raked to the catch basin and pumped to the harvester or hand-picked. On top of the harvester, shrimp are passed through a sieve (Figure 8). Water and pond debris are rejected off the harvester and shrimp are pushed further into a small water reservoir for transportation to a different area where further processing (euthanizing, weighing and storage) occurs.

Figure 7 – Fish pump draining a pond

Figure 8 – Harvesters sieve

After all shrimp are pumped out of the pond, they are then rinsed and put through a bath of ice and water to kill euthanize it. They are then weighed and stored in containers along with ice until transportation to processing building. Roughly 150 individuals are separated for individual weighing. The total yield value was over 9.1 tons and average survival was 60% (not including the pond with very high mortality). More shrimp pond production data will be presented further ahead in this report (10.1.1).

7.2 Pond Water Quality Management

For pond water quality a number of parameters were controlled: DO, Salinity, pH, Temperature, Ammonia and Turbidity. DO, salinity, pH and temperature were recorded thrice a day (0500-0530, 1400-1430, 1930-2000) in a YSI multiparametric probe (Yellow Spring Instrument Co., Yellow Spring, OH, USA). Off-schedule readings were performed every time it was considered necessary (e.g. algae crash, DO chronically low). Ammonia and turbidity were registered once every week. Ammonia was assed by running water samples from every pond through an ISE Multiparameter probe (Thermo Scientific™ Orion™ 4-Star Plus pH/ISE Benchtop Multiparameter Meter). Turbidity was registered by Secchi disk measurement (as mentioned previously). Control and interpretation of the variability of these parameters should present a good overview of the ponds dynamics and help identify potentially problematic situations such as low DO or water toxicity.

8. *T. carolinus* – Species Background

One of the species that will be used in nutrition trials through this internship period is the Florida pompano (*Trachinotus carolinus*) (Figure 9). It is a warm water marine carnivore fish found in the eastern Atlantic from the southeast US to Brazil (Main, 2007). In the Atlantic southern coast of the US, Florida pompano is especially important as a sport fishing species. CPMC hatchery facility has this species breeding as one of their ongoing tasks.

Figure 9 – Juvenile Florida pompano (*T. carolinus*)

Although having a history of capture, the first aquaculture production data only goes back to 2004 and FAO (FAO, 2017b) data indicates that by 2015 more than 80% of the global production (436 tons) came from aquaculture.

With a growing participation of aquaculture to the global production, these trials focus on the evaluation of substitution of fishmeal by soy-based diets. As in many species, simply substitution of fishmeal for plant-based meal has negative effects in both feed conversion and digestive track histology. Therefore, the trials conducted through this internship have the objective of further understanding how to fulfill *T. carolinus* nutrient requirements with plant-based ingredients and at the same time mitigate negative effects on growth performance and histology/physiology.

Further information on these trials will be presented further ahead in this report.

9. Florida Pompano Tank Culture

As previously described in tank culture of shrimp, the culture of *T. carolinus* at CPMC relied on RAS. The main difference between shrimp and pompano is that pompano required higher water volume (depth) as they occupy the whole water column, and shrimp mainly habit the bottom/sediment. Regular maintenance of RAS during nursery and trials and is done by daily syphoning solid waste in the water reservoir and weekly backwashing the systems.

Through this internship, Florida pompano culture was done with the single purpose of expanding our understanding of the nutrition of this species. For the same reason shrimp nutrient requirement trials are done in clear water systems, pompano trials were done in clear water systems. Fish for all trials were nursed for a differential periods of time, depending on their size as well as system availability. For one of the systems a high mortality occurred at stocking so the experiment had to be restarted. The second pompano experiment (described in 10.2.1) to be stocked was scheduled to run two weeks after the first but ended up only being stocked six weeks later. For the period when high or ongoing mortality was registered, all tanks of the system were treated with copper sulfate. Copper sulfate is a common general biocide in aquaculture. Particularly among marine teleosts.

Through all pompano trials, experiments were sampled once every two weeks. Pompano sampling consisted of weighing all individuals (group weight) to register growth in order to adjust feed inputs as well as monitor growth. For sampling, fish are captured from each tank and weighed. Before returning to the tank all fish are dip respectively in salt water and chloroquine solution and followed by a freshwater dip. Dips in high concentration are a useful procedure for small groups of fish although removal from culture tank (Roberts, 2012). In aquaculture chloroquine is commonly used as treatment for parasites such as *Amyloodinium ocellatum* and *Cryptocaryon irritans* (Noga, 2010). It is an expensive substance that is mainly used to treat malaria infections in humans. Although used to treat some parasite infections its therapeutic concentration is highly toxic for micro and macroalgae as well as other invertebrates (C.E. Bower, personal communication; Noga, 2010). Freshwater dips are a common practice to prevent ectoparasite disease outbreaks.

As freshwater (or salinity adjustment) is an effective therapy towards protozoans, Monogenea and some crustaceans (Langdon, 1992; Noga, 2010), it is common that farmers take advantage of this situation to prevent disease outbreaks. Particularly with species that can withstand the decrease needed. Florida pompano is a marine species with capacity to withstand some salinity drop. Therefore, in all experiments, salinity was dropped and fish grew at roughly 28.5 ppt saltwater.

9.1 Florida Pompano Tank Culture Water Quality Management

Water quality management for *T. carolinus* tank culture followed the same methods as shrimp tank culture. And as described before in this report (sub-chapter 6.1).

10. Nutrition Experiments Run

This chapter has the objective of providing a quick overview to all the trials that took place at CPMC during the period of this internship. This overview is composed by a short description in order to explain the relevance of these studies. All trials (except the one addressed in 10.2.2) are either published or being prepared for publication. Any result here presented has the sole objective of giving a better perception of the trial. The results and intellectual property are credited to the authors of the trials and publications, and not the author of this report.

10.1 *L. vannamei* Trials

For all shrimp trials, postlarvae were obtained from Shrimp Improvement System, Islamorada, Florida, USA, and subjected to the same nursing treatment. Six 6000L in recirculating system, with reservoir, biological filter supplemental aeration and recirculating pump, were used as nursery system (Figure 10).

Figure 10 – The six tanks (grey tanks on left side) used for PL nursing period

10.1.1 "Feed management and the use of automatic feeders in pond production of Pacific white shrimp *Litopenaeus vannamei*"

One of the most important factors for the success of an aquaculture farm is a careful management of feed. For its important weight in a farms expenses and impact in water quality, feed management is pivotal for the success of a farm. Feed management may be approached from different perspectives such as feed constitution or feeding system. Currently, shrimp farming relies in vegetable-based diets and automated feeding systems. Although automatic scheduled feeders, are not a new technology, they are one of the most common systems used of a variety of species, both fish and shrimp. However, scientific and technological progress allowed the development of more advanced systems. One of the most recent technologies to be used in shrimp farming is sound-feeding system. The sound-feeding system used in this research was provided by AQ1 (AQ1 Systems Pty. Ltd. Tasmania, Australia). AQ1 sonic-feeding systems are a recent technology that integrates water quality (underwater probe) and shrimp feeding demand (underwater hydrophone) data in real-time, allowing farmers to improve their feed management (Figure 11). However, for this internship no underwater probe was used, and feeders were turned and off according to individual DO checks. Also slightly different from the following figure, this experiment did not use a hydrophone hanging on a floater on the water surface, but rather a floating hydrophone connected to a small heavy piece on the bottom of the pond.

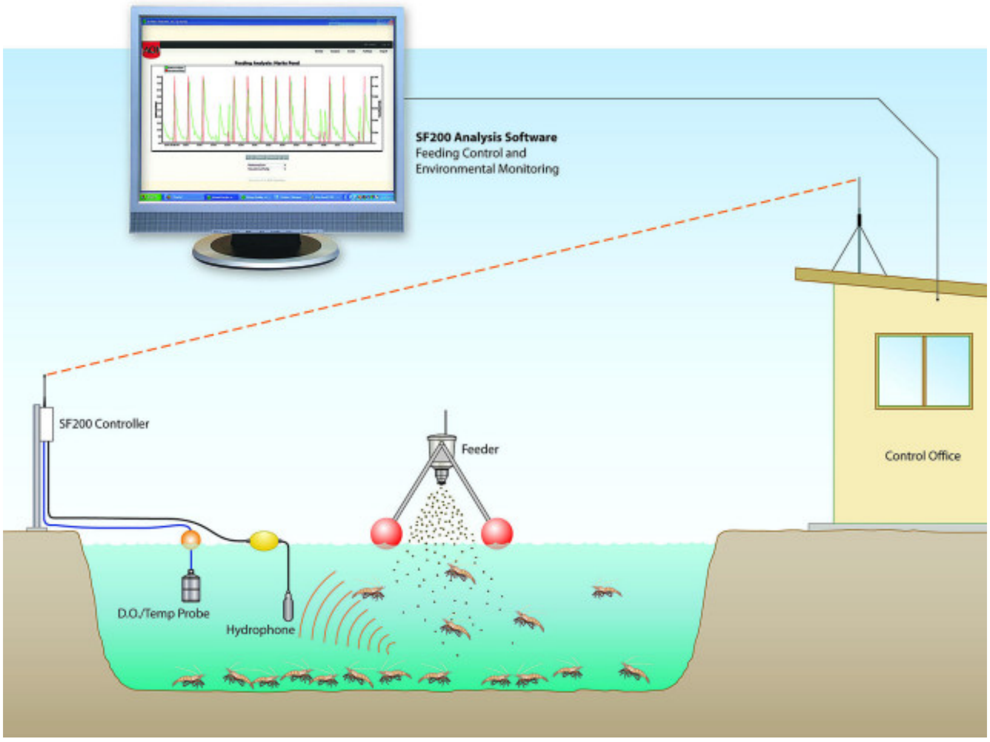

Figure 11 – AQ1 sonic-feeding squeme (Source: AQ1 Systems)

The objective of this research is comparing four different feeding systems and regimes (hand-feeding, solar timer-feeder 115% sound-feeding system, solar timer-feeder, and hand-feeding) through water quality (temperature, pH, salinity, and DO) and growth factors (e.g. final biomass, individual weight, FCR, weight gain) as well as farm running costs (i.e. electricity and feed costs).

A standardize feeding protocol (SFP) for feed amounts which has been developed over time served as the reference. The feed input for the four treatments are as follows: Treatment 1 – hand-fed SFP; Treatment 2 – Solar timer feeding SFP 115%; Treatment 3 – Solar timer feeding SFP 130%; Treatment 4 – sonic-feeding up to 15 kg/day. Each treatment was applied to four replicates (ponds). Automatic feeders for treatment 2, 3 and 4 were set at the end of the dock closer to the center of the pond. Another important note is the fact that automatic feeders have a very limited capacity to spread the feed across the pond (usually only about a 1.5 m radius around the feeder), comparing to hand-feeding management. However, size uniformity does not seem to be negatively affected. Which is similar to what happens in many other groups and species.

Treatment 1 ponds were hand-fed twice a day (0800 and 1600). For wider feed distribution, half of the feed amount was thrown from each end of the pond. Both treatment 2 and 3 (solar-powered timer-feeders) distributed the daily feed equally through six meals (0800, 1000, 1200,1400, 1600, 1800). Feed input was calculated for each treatment (except sonic-feeders) based on the weekly sampling data on shrimp growth. For SFP calculation, it was estimated an overall 75% mortality after 16 weeks, which estimates a 2% every week. Ponds under sonic-feeding treatment were allowed to distribute feed on demand (shrimp feeding response), but limited to 15 kg a day. Shrimp in the ponds were fed "Semi intensive SI-35%P 7%L 2.4 mm" by Zeigler (Zeigler Bros Inc., Gardners, PA, USA), which is a commercial plant based diet. This trial was planned to be run for 16 weeks but good growth performance anticipated harvest and it was run for just 13 weeks.

The results of this study indicate significant differences between all automatic feeding systems and hand-feeding management. Within automatic feeding systems, sonic-feeders showed the best growth performance. These differences are found in yield (kg/ha), individual weight, weight gain, week growth, biomass, feed cost, value, and partial income. However, FCR and survival didn't show any significant difference. Due to high mortality in one of the treatment 4 ponds, data from that replicate was not used for statistical purposes.

The title of the future publication is expected to be the same as this sub-chapter, and the authors are Carter Ullman, Melanie Rhodes, and D. Allen Davis, from School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University.

10.1.2 "The effects of feed leaching on the growth and performance of Pacific white shrimp *Litopenaeus vannamei* in a green-water tank system"

The leaching of nutrients from feed is a primary issue in fish and shrimp nutrition in aquaculture. Contrary to terrestrial livestock feeds, aquatic animals feed will not be completely stable in water and as soon as it reaches water it starts to leach its nutrients. As feed is the main production cost of an aquaculture farm, leaching of nutrients from feed is an economically negative factor as much as a biological inconvenience. Particularly for protein leaching, it will increase the nitrogen pollution in water (toxic for fish and shrimp). Feed leaching in shrimp species is particularly evident as shrimp externally masticate their feed and eat relatively slowly, meaning feed is in the water for an extended period of time. For shrimp reared in low salinity conditions it is also

The purpose of this study was to examine the effects of leaching on the nutritional qualities of commercial shrimp feed and the growth of Pacific white shrimp *L. vannamei*.

This trial was performed in an outdoor semi-recirculating 24 (800 L) tank green water system for 8 weeks. This system also included an 800 L water sump. The water source of this trial was a production pond, and it was pumped daily for two hours into the system (5% daily water exchange). Each tank was stocked with 30 shrimp (35 shrimp/m^2) hand sorted to uniform size (average 0.3 g/individual). Water quality management was performed according to previous description in this report (6.2).

Six treatments were used to examine the effects of leaching after 0, 0.5, 1, 2, 4, and 6 hours. The diets formulated for this trial were based in a commercial extruded shrimp diet (35% concentrate protein and 7% lipid) produced by Zeigler, Inc (Gardners, Pennsylvania, USA). Feed leaching was achieved by floating drying trays (with window screen in the bottom) on fresh water for the correspondent amount of time of each treatment. Each drying tray was filled with 1 kg of feed spread evenly. Once removed from water the feed was then dried by, respectively, placing the trays in the sun for two hours with a blowing fan across the feed, and place the trays in a fan ventilated oven for 24 hours.

Results for this trial indicate a steep reduction in methionine after a short period of time (0.5 hours) but did not further decrease. Taurine showed a negative trend relative to the leaching period in the amino acid analysis. Potassium, sodium, and sulfur also showed negative trends in the mineral analysis. The 0 and 0.5 hour treatments produced shrimp that were significantly bigger than all other treatments.

The results of this study indicate that feed leached for more than 0.5 hours can have a negative impact on the nutritional profile of the feed and a significant impact on the growth of the shrimp.

The title of the future publication is expected to be the same as this sub-chapter, and the authors are Carter Ullman, Melanie Rhodes, and D. Allen Davis, from School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University.

10.1.3 "Utilization of crystalline amino acids by Pacific White Shrimp *Litopenaeus vannamei*"

Substitution of fishmeal in fish and shrimp diets with less costly protein sources has received considerable attention as a mechanism to reduce cost and build a more sustainable industry. However these new protein sources very rarely have a good essential amino acid (EAA) balance, therefore enhancing the necessity of defining EAA requirements and adjusting diets. An ideal protein balance may be achieved by either mixing different protein sources or adding crystalline amino acid (CAA). CAA supplementation is common way of meeting a species EAA requirements. However, their use by aquaculture industry has been slowly adopted as there are still concerns with regard to the efficacy, particularly in shrimp (NRC, 2011). Therefore, the pertinence of this study was to determine the efficacy of CAA use in practical *L. vannamei* diets.

Basal diets included the following protein balance: 5% fishmeal, 37% soybean and 5% gelatine. The diets designed for this trial were constituted by 30% protein and 6% lipid. In experimental diets the intact protein component was reduced to 28, 26, 24 and 22% in four diets, and also reduced to 28, 26, 24 and 22% with further addition of CAA by 2, 4, 6 and 8%, respectively. This study was performed in an indoor 44 square clear water tanks recirculating system with one reservoir, biological filter, supplemental aeration and circulation pump (Figure 12). Each tank was stocked with 15 shrimp and the trial was conducted through seven weeks.

The results of this trial indicate that shrimp don't show better response to CAA supplemented diets, but rather to intact protein. High variation in growth performance and poor FCR eventually determined that this trial would be rerun to confirm the results.

The title of the future publication is expected to be the same as this sub-chapter, and the authors are Anneleen Swanepoel, D. Allen Davis, G. A. Harsha S. Chaturanga, and Xuan Qiu, from School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University.

Figure 12 – 42 tank recirculating system used in CAA trial

10.1.4 Evaluation of growth performance of Pacific White Shrimp *L. vannamei* fed salmon meal and amino salmon P60 in substitution of anchovy meal in green water system

This trial was performed to evaluate the utilization of salmon meal and amino salmon P60 meal on growth performance of the Pacific White shrimp, *Litopenaeus vannamei* in reared in green water system. For this trial, practical diets with growing levels of dried bakers yeast (DBY) (0, 10.55, 15.85 and 21.10%) were used to replace anchovy meal (20, 10, 5, and 0%). At the same time, amino salmon P60

(60% protein) meal was used to replace 50% and 100% of anchovy meal (Diet 5 and 6).

This trial was performed in this same system as the leaching feed trial (at different times) under similar conditions. However, this trial was run through 10 weeks. Water quality management was also performed according to previous description in this same report (6.2).

At the time of delivery of this report no results had yet been achieved for the trial had just been finished.

The authors of this study are Jingping Guo, Melanie Rhodes, and D. Allen Davis, from School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University.

10.2 *T. carolinus* Trials

For all three pompano trials, the fingerlings were obtained from Proaquatix, Vero Beach, Florida, USA. For all trials fish were subjected to a nursing period in indoor recirculating systems and commercial feeding until suitable size.

10.2.1 "Effects of soybean meal replacement with fermented soybean meal on growth, serum biochemistry and morphological condition of liver and distal intestine of Florida pompano *Trachinotus carolinus*"

This trial is first set in the global trend of substitution of fishmeal (FM) (or other animal meal) for plant-based protein meal. Juvenile *T. carolinus* has shown good performance with combination of plant-based protein and 15% animal meal (Rossi and Davis, 2012). One of the plant protein sources is soy bean meal (SBM), which has been used in combination with poultry by product meal (PBM) and/or meat bone meal (MBM) and taurine to reduce FM component in practical *T. carolinus* diets (Rossi and Davis, 2012; Rossi and Davis, 2014; Rhodes et al, 2017b). Corn protein concentrate (CPC) is a non soy-based product used in practical diets for pompano for its ability to balance SBM amino acid profile, free of anti nutritional factors (ANF's) and rich in methionine and cysteine (Phillips and Sternberg, 1979, Gatlin *et al.*, 2007, Robinson and Li, 2008, Khalifa *et al.*, 2017). Produced by fat trimming of pork carcass, porcine meal (PM) may be included in plant-based fish and shrimp diets

(Hernández et al, 2010, Hernández et al, 2008, Wang et al, 2012) for it compensates the limited hydroxyproline levels in plant protein products (Aksnes et al, 2008, Wu et al, 2008). Hydroxyproline also seems to play an important gustatory response to some species, in their natural pH (Marui et al, 1983, Hara, 2012).

This study has the objective of providing information of different soy source protein fortified with PM and various inclusion levels of CPC to improve efficiency of soy based diets for *T. carolinus*. The study focuses on growth, body composition and distal histopathology.

The diets used in this trial were produced to be iso-nitrogenous and iso-lipidic (40% protein and 8% lipid) using poultry meal (PBM, Griffin Industries Inc), de-hulled solvent extracted SBM, advanced soy product (ASP, Nutrivance) fermented soybean meal (FSBM, PepSoyGen, Nutrafrena), corn protein concentrate (CPC, Empyreal 75th) and porcine meal (PM, Innomax MPI, Sonac) as the dietary protein sources (Novriadi, 2017). The reference diet used in this study was a 15% poultry meal produced with respectively 150, 495 and 70 g kg^{-1} of PBM, SBM and CPC.

This trial was conducted in an indoor recirculating system (Figure 8) composed by 12 circular tanks and one reservoir, biological filter, supplemental aeration and circulation pump (Figure 13). The trial was run through 56 days and feed adjustments were calculated after each sampling. Fish sampling for this experiment was performed as described before in chapter 9.

Figure 13 – 12 tank recirculating system used in soybean experiment

Body composition analyses were performed both on random fish before stocking, and random fish from every tank upon termination of the trial. Fish were blended in a mixer as described by Association of Official Analytical Chemist (AOAC). All body analyses were performed by a third party. For histology, three fish were selected for histological analysis and euthanized in a MS222 solution (Ethyl 3-aminobenzoate methanesulfonate salt, Salt) and once separated, fish distal intestine were preserved in Bouin solution for 20h at room temperature and then transferred to 70% ethanol solution.

The title of the future publication is expected to be the same as this sub-chapter, and the authors are Romi Novriadi, Melanie Rhodes and D. Allen Davis, from School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University. This research is currently waiting for publication.

10.2.2 The Effects of Incorporation of Different Levels of Hydroxyproline on Growth and Body Composition in Florida pompano *T. carolinus* Experimental Diets

The substitution of fishmeal for plant protein sources has been one of the major trends in aquaculture for the last decades. Much progress was done as far as removal of anti-nutritional factors are concerned. But it is also important to identify the fishmeal nutrients that are missing in plant sources and may further improve fish growing performance. Although anti-nutritional factors explain the big performance difference between fishmeal and plant diets, other factors could be of importance (Aksnes et al., 2008).

Hydroxyproline (hyp) is the product of proline hydroxylation. It is an important constituent of collagen, and can be found in fish fillet or bone tissue (such as spine). It is present in both fishmeal and plants used as feed, but its levels are much higher in fishmeal and several works have show performance improvements when using hyp rich byproducts (Aksnes et al., 2006).

The objective of this trial is to study the effects of incorporation of different levels of hydroxyproline in *T. carolinus* experimental diets in growth and body composition. Five experimental diets were formulated with 0%, 0.5%, 1%, 2%, 4% of hydropxyproline. Each tank was stocked with 15 fish (average 21.29 g/individual). This trial was run for 62 days in a 24 tank RAS system (Figure 14). For this trial, fish were sampled as described earlier in chapter 9.

Figure 14 – 24 tank recirculating system used in this experiment

The authors of this study are Romi Novriadi, Melanie Rhodes, Guillaume Salze and D. Allen Davis, from School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University. High mortality rates in the first two attempts to stock the system and further mortality through the trial (third stocking attempt), made the results improper for publication. The data collected in this trial will be used as a perspective for future studies.

10.2.3 Growout Trial for Florida pompano *T. carolinus* fed different commercial diets

The objective of this experiment was to compare the growth performance of juvenile *T. carolinus* fed three commercial diets and growth performance was assessed. The three floating feeds used in this trial were respectively Zeigler FF Silver 40/10 3.0 mm (Zeigler Bros Inc., Gardners, PA, USA), Rangen Extra 400 3.0mm (Rangen Inc, Idaho, USA) and Skrett ing Steelhead 3.5mm (Skretting USA, Tooele, UT, USA). This trial was performed on the same system used for shrimp nursery (Figure 10). Tanks were stocked with 20 fish and each treatment had two replicates.

The authors of this study are Melanie Rhodes and D. Allen Davis, from School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University.

11. Conclusion

The opportunity to intern at Auburn University's nutrition team was a very good opportunity to develop my practical training in aquaculture field. It allowed me to improve and learn new technical skills, as well as developing my team working capacity. This internship also improved my communication skills in English language by integrating a team of international students in an environment where clear communication is a pivotal key to final success.

Technically, this internship allowed me to better understand the standard procedures of aquaculture systems in both production and research environment. The daily contact with the challenges aquaculture production and research present has allowed me to develop my knowledge in detecting simple abnormal conditions. Especially in the outdoor ponds, experience gained through this internship allowed me to generally understand the ponds natural dynamics by reading simple water quality indicators (DO, salinity, pH and temperature) or just observing the pond (e.g. color of surface). The daily contact and understanding with ponds dynamics and the way feeding and water quality interact is perhaps the most important practical outcome of this internship as in both research trials and production farms it affects the survival and wellness of shrimp.

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